Appl. No. 10/004,115
Amdt. dated February 6, 2004
Reply to Office Action of November 19, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A polynucleotide sequence having:

a) a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1;

b) a polynucleotide sequence that hybridizes[[, under stringent conditions,]] with a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1 to form a hybrid under high ion concentrations of 900mM sodium chloride and 90mM sodium citrate at 65°C and the hybride formed is maintained as a hybrid after being kept at 65°C for 30 minutes under a low ion concentration of 15mM sodium chloride and 1.5mM sodium citrate, the amino acid sequence being an amino acid sequence of ~~a protein~~ an enzyme capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanotate; ~~or~~

c) ~~a~~ an isolated polynucleotide sequence represented by SEQ ID NO: 2,

d) a polynucleotide coding for an amino acid sequence of SEQ ID NO: 1 having additional 6 amino acids of Trp-Ile-Ser-Thr-Lys-Leu at the C-terminal of the amino acid sequence;

e) a polynucleotide sequence having 80% or more sequence identiyy with the polynucleotide sequence coding for an amino acid sequence of SEQ ID NO: 1, or

f) a polynucleotide sequence that hybridizes with a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1 to form a hybrid under high ion concentrations of 450 to 900mM sodium chloride and 45 to 90mM sodium citrate at 65°C and

the hybride formed is maintained as a hybrid after being kept at 65°C for 30 minutes under a low ion concentration of 15 to 300mM sodium chloride and 1.5 to 30mM sodium citrate and 0.1 to 1.0 wt% of SDS, the amino acid sequence being an amino acid sequence of an enzyme capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

Claim 2 (original): A DNA construct comprising a promoter in operative linkage with the polynucleotide sequence as defined in Claim 1.

Claim 3 (original): A recombinant vector containing the polynucleotide sequence as defined in Claim 1 or 2.

Claim 4 (currently amended): A transformant having
the DNA construct as defined in ~~claim 2, or~~
~~the vector as defined in Claim 3~~ Claim 2.

Claim 5 (original): A transformant according to Claim 4, wherein the transformant is a microorganism.

Claim 6 (original): A transformant according to Claim 5, wherein the microorganism is *E. coli*.

Claim 7 (original): A process for producing a transformant, which comprises the step of introducing the recombinant vector as defined in Claim 3 into a host cell.

Claim 8 (currently amended): A transformant having the polynucleotide as defined in ~~claim~~ Claim 1.

Claim 9 (currently amended): A recombinant vector containing

A) a polynucleotide construct as defined in Claim 1, and

B) a polynucleotide coding for an enzyme capable of converting oxidized ß-nicotinamide-adenine dinucleotide phosphate into a reduced form, wherein the enzyme is glucose dehydrogenase.

Claim 10 (canceled)

Claim 11 (currently amended): A transformant having the vector according to Claim 9 ~~or 10~~.

Claim 12 (original): A transformant according to Claim 11, wherein the host is a microorganism.

Claim 13 (original): A transformant according to Claim 12, wherein the microorganism is *E. coli*.

Claim 14 (currently amended): A transformant having

A) the polynucleotide as defined in Claim 1, and

B) a polynucleotide coding for an enzyme capable of converting oxidized ß-nicotinamide-adenine dinucleotide phosphate into a reduced form, wherein the enzyme is glucose dehydrogenase.

Claim 15 (original): A protein having:

i) an amino acid sequence of SEQ ID NO: 1;

ii) an amino acid sequence encoded by a polynucleotide sequence that hybridizes under stringent conditions with a polynucleotide sequence of SEQ ID NO: 2 coding for an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; or

iii) an amino acid sequence of SEQ ID NO: 1, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

Claim 16 (currently amended): A process for producing (S)-4-halo-3-hydroxybutanoate, which comprises reacting 4-halo-3-oxobutanoic acid ester with the protein as defined in ~~claim~~ Claim 15, a transformant, which produces said protein or a treated product thereof.

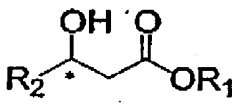
Claim 17 (original): A process according to Claim 16, which comprises allowing the coexistence of an enzyme capable of converting the oxdized ß-nicotinamide-adenine dinucleotide phosphate into a reduced form.

Claim 18 (original): A process according to Claim 17, wherein the enzyme capable of converting an oxidized ß-nicotinamide-adenine dinucleotide phosphate into a reduced form is a glucose dehydrogenase.

Claim 19 (currently amended): A process according to ~~claim~~ Claim 17, wherein the 4-halo-3-oxobutanoic acid ester is contacted with the transformant as defined in any one of Claims 11 to 14 or a treated product thereof.

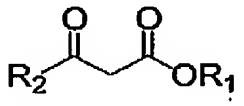
Claim 20 (currently amended): A process according to ~~claim~~ Claim 16, 17, 18 or 19, wherein the

4-halo-3-oxobutanoic acid ester is represented by a formula (1):



(1),

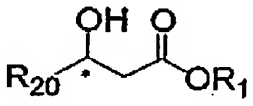
wherein R_1 represents an alkyl group, and R_2 represents a methyl group which is substituted with a halogen atom, which process comprises reacting 4-halo-3-oxobutanoic acid ester of formula (2):



(2),

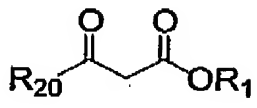
wherein R_1 and R_2 represent the same as defined above.

Claim 21 (currently amended): A process for producing an optically active 3-hydroxybutanoic acid ester of formula (1a):



(1a),

wherein R_1 represents an alkyl group, and R_{20} represents a methyl group which may be substituted with a halogen atom, which process comprises reacting 3-oxobutanoic acid ester of formula (2a):



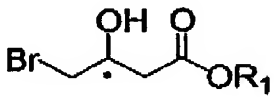
(2a),

wherein R_1 and R_{20} represent the same as defined above, with **[[a]]** whole cells of a microorganism or a treated product thereof, which microorganism belongs to *Penicillium citrinum, Cryptcoccus humicolus,* or *Bacillus alvei* and is capable of asymmetrically reducing the oxo group at 3-position of the compound of formula (2a) to corresponding 3-hydroxy group.

Claim 22 (original): A process according to claim 21, wherein R_2 represents a halomethyl group.

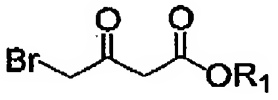
Claim 23 (currently amended): A process according to claim 21 or 22, wherein the ~~microoranism~~ microorganism is a strain selected from the group of *Penicillium citrinum* (IFO4631), *Cryptcoccus humicolus* (IFO1527), and *Bacillus alvei* (IFO3343t).

Claim 24 (original): A process for producing an optically active 4-bromo-3-hydroxybutanoate of formula (1b):



(1b),

wherein R_1 represents a (C2-C8) alkyl group, which process comprises reacting 4-bromo-3-oxobutanoate of formula (2b):



(2b),

wherein R_1 represents the same as defined above, with an enzyme having:

iv) an amino acid sequence of SEQ ID NO: 34;

v) an amino acid sequence encoded by a polynucleotide sequence that hybridizes, under stringent conditions, with a polynucleotide sequence of SEQ ID NO: 34, wherein said amino acid sequence is an amino acid sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; and

vi) an amino acid sequence of SEQ ID NO: 3, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being an amino acid sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxbutanoate.

Claim 25 (original): A process for producing 4-cyano-3-hydroxybutanoic acid, which comprises reacting 4-bromo-3-hydroxybtanoic acid ester with a metal cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earth metal halogenide.

Claim 26 (currently amended): A process according to ~~claim~~ Claim 25, which further comrprises the step of reacting the 4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce 4-cyano-3-hydroxybutanoic acid alkyl ester.

Claim 27 (currently amended): A process according to ~~claim~~ Claim 25 or 26, wherein the alkaline earth metal hydroxide is calcium hydroxide, and the alkaline earth metal halogenide is calcium chloride.

Claim 28 (currently amended): A process according to ~~claim~~ Claim 25, wherein the ~~4-bromo-3-hydroxybtanoic~~ 4-bromo-3-hydroxybutanoic acid ester is (C1-C8) alkyl 4-bromo-3-hydroxybutanoate, and the dialkyl sulfate is dimethyl or diethyl sulfate.

Claim 29 (currently amended): A process according to ~~claim~~ Claim 25 or 26, wherein the 4-bromo-3-hydroxybutanoic acid and ~~4-cyano-3-hydroxybtuanoic~~ 4-cyano-3-hydroxybutanoic acid are optically active compounds.

Claim 30 (currently amended): A process according to ~~claim~~ Claim 25 or 26, wherein the 4-bromo-3- hydroxybutanoic acid is (S)-4-bromo-3-hydroxybutanoic acid ester and ~~4-cyano-3-hydroxybtuanoic~~ 4-cyano-3-hydroxybutanoic acid is (R)-4-cyano-3-hydroxybutanoic acid.

Claim 31 (currently amended): A process for producing (R)-4-cyano-3-hydroxybutanoic acid, which comrpises

producing (S)-4-bromo-3-hydroxybutanoic acid ester by asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester, and

reacting ~~(S)-4-bromo-3-hydroxybtanoic~~ (S)-4-bromo-3-hydroxybutanoic acid ester with a metal cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earthmetal halogenide.

Claim 32 (currently amended): A process according to ~~claim~~ Claim 31, wherein the asymmetrical reduction is conducted by a microorganism or treated product thereof capable of asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester to (S)-4-bromo-3-hydroxybutanoic acid ester.

Claim 33 (currently amended): A process according to ~~claim~~ Claim 32, wherein the microorganism is a microorganism belong to *Penicillium citrinum*.

Claim 34 (currently amended): A process according to ~~claim~~ Claim 31, 32 or 33, wherein (S)-4-bromo-3- hydroxybutanoic acid ester and 4-bromo-3-oxobutanoic acid ester are (C1-C8) alkyl ester.

Claim 35 (currently amended): A process according to ~~claim~~ Claim 33, wherein the microorganism is a strain *Penicillium citrinum* (IFO 4631).

Claim 36 (currently amended): A process according to any one of ~~claim~~ Claim 31 to 35, wherein the alkaline earth metal hydroxide is calcium hydroxide and the alkaline earth halogenide is calcium chloride.

Claim 37 (currently amended): A process according to ~~claim~~ Claim 31, which further comprises the step of reacting (R)-4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce (R)-4-cyano-3- hydroxybutanoic acid ~~with dialkyl sulfate to produce (R)-4-cyano-3-hydroxybutanoic acid alkyl ester~~.

Claim 38 (currently amended): A process according to ~~claim~~ Claim 32, wherein the alkyl group of the dialkyl sulfate is a methyl or ethyl group.

Claim 39 (new): A transformant having the vector as defined in Claim 3.

Claim 40 (new): A recombinant vector according to Claim 9, wherein the enzyme in B) is glucose dehydrogenase derived from *Bacillus megaterium*.

Claim 41 (new): A transformant according to Claim 14, wherein the enzyme is glucose dehydrogenase derived from *Bacillus megaterium*.